ANTI-MULLERIAN HORMONE: POTENTIAL ASSOCIATION WITH FERTILITY IN EWES

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ABSTRACT

Two flocks of Rambouillet ewes, with and without the Booroola gene, were sampled for AMH concentrations and fertility characteristics. The total sample population consisted of 307 ewes. The objective of this study was to estimate the effect of FecB genotype and BW on AMH concentrations, estimate the difference in AMH concentrations between pregnant and non-pregnant ewes and to estimate the difference in AMH concentrations between ewes carrying single v.s. multiple fetuses. Sheep with one copy of the FecB gene showed reduced AMH concentrations when compared to ewes that had no copies of the FecB gene. Genotype had a significant effect on AMH concentrations prior to exposure and at mid gestation. Fetal count had a significant source of variance in group 2. Genotype had a significant effect on AMH concentrations in both flocks. Fetal count was shown to be a significant source of variance of AMH concentrations in one group.

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INTRODUCTION

As the population of the world increases and the demand for food protein increases, the need for producers to increase production is extremely important. One cost-effective way to increase production is through improvements in reproductive efficiency. Most mammals in temperate and northern climates breed at a specific time of year so that their offspring will be born in the spring. This is done in order to increase the offspring's chance of survival. For sheep, in order to lamb in the spring, this means that a ewe must breed in fall. Most western range commercial producers keep their replacement females from the spring lambing and breed them roughly 18 months later.

A ewe is most reproductively efficient between the ages of 3 and 6 years-of-age (Dickerson et al., 1975). The reproductive efficiency of ewes then starts to decline around year 7 years-of-age (Dickerson et al., 1975). Because of this, a producer will not be able to begin to estimate potential reproductively efficiency in a ewe until age 3. Shown in previous studies, Anti-Mullerian hormone is present in prepubertal ewe lambs and could help producers in making management decisions when selecting for replacement expected fertility at the first mating (Lahoz et al., 2012). Anti-Mullerian Hormone is shown to be present in the plasma of prepubertal ewe lambs and plasma concentration is related to the occurrence of ovulation in ewe lambs after being treated with an ovarian stimulant (Lahoz et al., 2012). According to some authors the number of offspring obtained per lambing season is more important than the weight gained (Petrovic et al., 2012).

Booroola Merino sheep have been crossed with Rambouillet sheep to help improve reproductive rates (Willingham et al., 2000). The Booroola (FecB) gene could have a possible relationship with AMH concentrations. Half of the ewes in this study have one copy of the FecB gene and the other half are wild-type non-carriers of the gene.

OBJECTIVE STATEMENT

The objectives of this study are to 1) estimate the effect of FecB genotype and BW on AMH concentration 2) estimate the difference in AMH between ewes that are pregnant and not pregnant, and 3) estimate the difference in AMH between ewes carrying single and multiple fetuses.

LITERTURE REVIEW

Anti-Mullerian Hormone (AMH)

Anti-Mullerian Hormone is found in fish, reptiles, birds, and mammals. In mammals it is present in both males and females. Anti-Mullerian hormone could potentially have an association with fertility characteristics such as puberty, pregnancy, earliness of conception, and fecundity in Rambouillet ewes either with or without the Booroola (FecB) gene.

Anti-mullerian hormone is a member of the transforming growth factor beta superfamily and is a homodimeric (meaning that it consists of two identical subunits) disulfide linked glycoprotein (La Marca et al., 2006). The AMH gene is located on the short arm of chromosome 19 in humans. During male embryonic development AMH is present and induces the regression of the Mullerian ducts before birth (Visser et al., 2006). This process then allows the wolfian duct system to develop, giving rise to many of the anatomical structures of the male reproductive system. In the female embryo the mullerian ducts continue to develop, giving rise to many of the anatomical structures of female reproductive system (Bruce Carpenter, personal communication). Later, it continues to be produced by the sertoli cells of the male testis from testicular differentiation up to puberty, and to a much lesser degree in ovarian granulosa cells from birth up to menopause (La Marca et al., 2006). As in other species, AMH is present in male mice
and rats, but AMH is not present in female mice and rats before birth (Durlinger et al., 2002). The lack of AMH expression, in females, guarantees the development of the internal reproductive tract (Durlinger et al., 2002). Shortly after birth AMH expression is detected in the granulosa cells of growing follicles (Durlinger et al., 2002). In both humans and rodents, AMH expression begins in the granulosa cells of primary follicles and is highest in preantral

and small antral follicles (Campbell et al., 2012). This is shown to prevent the recruitment of primary follicles and therefore prevents the premature exhaustion of the follicular reserve (Estienne et al., 2015). Anti-Mullerian hormone seems to be stable until adulthood. When AMH concentration decreases, it is a sign of follicle reserve exhaustion (La Marca et al., 2006). All females are born with a finite number of ovarian follicles and it has been shown that the quality of these follicles declines with age (Vissser et al., 2006). Again, AMH is strongly expressed in the sertoli cell from testicular differentiation up to puberty and to a much lesser degree in the granulosa cells from birth up to the end of the reproductive life. Anti-Mullerian hormone seems to be steady until adulthood when it decreases this is a sign of follicle exhaustion (La Marca et al., 2006). The ovarian reserve is constituted by the size of the ovarian follicle pool and the quality of the oocytes within (Visser et al., 2006). In the cow, AMH plasma concentration is positively correlated to the number of antral follicles from 3 to 7 mm in diameter, which are the main target for superovulatory treatments (Lahoz et al., 2012). The positive correlation between AMH and antral follicle count (AFC) and the number of healthy follicles and oocytes within the ovaries shows some evidence that there is a direct relationship between AMH and fertility (Lahoz et al., 2012). The expression of AMH is not always distributed evenly. In some follicles expression is the highest in the granulosa cells surrounding the antrum and the oocyte (Durlinger et al., 2002). Anti-Mullerian hormone has not been found in the primordial follicles, theca cells, oocytes, or the interstitium (Durlinger et al., 2002) In Transgenic mice, the presence of AMH during the fetal period is detrimental to ovarian development (Durlinger et al., 2002). Development of the ovary seems to be unaffected in mice with the lowest serum AMH concentrations (Durlinger et al., 2002).

According to La Marca et al., 2005 there were no differences in AMH levels during pregnancy in humans. During the follicular phase of the menstrual cycle, AMH levels were 1.9 ± 0.5 ng/ml (La Marca et al., 2006). Anti-Mullerian Hormone concentrations are not correlated with age (p= -0.19; NS) or live weight (p= -0.08;). However, AMH concentration was correlated with occurrence of ovulation in response to equine chorionic gonadotropin (eCG) (p=0.42; P<0.0001), but not with the number of ovulations when only comparing the ovulating ewes (p=0.08;) (Lahoz et al., 2012). Non-ovulating ewes had lower plasma concentrations than ovulating ewe lambs with 1, 2, or ≥3 ovulations (all P< 0.0004). However, no differences in AMH concentrations between the different groups of ovulating ewe lambs was observed (Lahoz et al., 2012). Also, the fertility of adult ewes at first, at second and after both consecutive service periods was correlated with their AMH concentrations in the prepubertal phase (r = 0.34, P< 0.01: r=0.33, P<0.01; r=0.36; P< 0.01, respectively)

Lahoz et al. (2012) developed a cutoff point of plasma AMH that could be used as a screening test for predicting the future ability of ewes to get pregnant at first mating. This was reported to 97 mg/ml during the prepubertal phase. The fertility of the first mating of ewes with AMH concentrations equal to or higher than 97mg/ml before puberty were 34.8 percent higher than ewes with lower AMH concentrations (P<0.001) (Lahoz et al. 2012). Similarly, the fertility at second mating or after both consecutive service periods was also higher in ewes with AMH concentrations, which met its cutoff value.

Ovarian Function

The ovaries respond to gonadotropins with both functional and structural changes (Inskeep, 2002). When exposed to gonadotropic stimulation, certain follicles increase in size and secrete sufficient amounts of estradiol (Inskeep, 2002). Increasing estradiol feeds back to the brain triggering estrus. Estradiol concentrations signal the brain that the mature follicles are present (Inskeep, 2002). Mature follicles are .4 to .7 cm in size when estradiol reaches peak in sheep reproduction. At peak estradiol production, Gonadotropin releasing hormone (GnRH) is released causing a massive release of LH (LH surge) and initiates the release of mature ova (ovulation) (Inskeep, 2002). The ovarian reserve is the size of the ovarian follicle pool and the quantity of oocytes with-in those follicles (Visser et al., 2006). The ovarian reserve declines with age and results in the decrease of a female's reproductive function (Visser et al., 2006).

AMH Serum Concentrations and Relationship to Antral Follicle Count

Anti-Mullerian Hormone serum concentrations are highly variable among nulliparous young adult cattle (Scheetz, 2010). The high variations of AMH concentration were reported among animals, rather than within individuals (Scheetz, 2010).

Anti-Mullerian hormones levels were relatively unchanged on a day to day basis during reproductive cycles of dairy cows, mice, and women (Scheetz, 2010). In contrast, AMH concentrations are slightly lower during the luteal phase compared with the follicular phase of the menstrual cycle in women. According to Scheetz (2010) the alterations in serum AMH during the cycle were minimal. Antral follicle count (AFC) is the number of follicles growing during the ovulation wave. Antral follicle count was positively associated with

AMH concentrations levels and is a good indicator for high, medium, and low AFC. Antral follicle count could possibly be associated with the fertility predictor's made using AFC without actually analyzing AFC in cattle, a producer can simply look at AMH levels prior to ovulation (Scheetz, 2010). Serum AMH concentrations from 8 days prior to ovulation to the day of ovulation remain unchanged ($P>0.72$) during the ovulatory follicular wave. Concentrations of AMH did not change with AFC groups ($P>0.20$) during the 6 to 8 days prior to ovulation, but were ~6 to 2 fold greater ($P<0.01$) in animals in the high and medium AFC groups compared with animals in the low AFC group (Scheetz, 2010).

The role of AMH in Human In-vitro Fertilization

As natural fertility begins to decrease after the age of thirty, many women are faced with the problems of becoming pregnant due to a decrease in ovarian reserve. Ovulation reserves are a combination of both the quantity and quality of the ovarian follicle pool (Broer et al., 2007). The number of primordial follicles left at a given age is an important indicator for ovarian exhaustion and can be an indicator for reproductive events, such as menopause (Broer et al., 2007). Counting the primordial follicles is not possible; and it has been shown that antral follicle count is strongly related to the size of the primordial follicles (Broer et al., 2007). An ideal ovarian test should show a sustainable percentage of IVF-indicated cases with a zero chance of becoming pregnant in a series of treatments because of the diminished ovarian reserve (Broer et al., 2007). These cases would be denied acceptance to the program because of a high burden on the patient and disappointing results (Broer et al., 2007). With this test, high cost of treatments with little to no results would be eliminated (Broer et al., 2007). However, a problem that Broer (2007) faced was a 10% to 20% false negative rate, but had a 70% to 80% positive test rate (Broer et al., 2007).

Booroola Gene (FecB)

The FecB gene was first discovered in a strain of Merino sheep known as the Booroola Merino (Willingham et al., 2000). The Booroola sheep were found to have undesirable phenotypic characteristics such as slow growth rate and smaller mature size (Willingham et al., 2000). Booroola sheep are known for high ovulation rates and prolificacy, which is due to a limited number of linked genes or by a single autosomal gene, known as the FecB Gene (Abella et al., 2005). Boorlooa Merino sheep were crossed with breeds in the United State, such as the Rambouillet, to increase reproductive rates (Willingham et al., 2000). Rambouillet Booroola cross ewes that are believe to carry one copy of the FecB gene had a 59% increase in ovulation rate when compared to straight Rambouillet ewes (Willingham et al., 2000). Prolificacy is a major parameter for the economic impact of the sheep industry (Abella et al., 2005). The Booroola gene (FecB), increases ovulation rate and litter size in sheep and is inherited as a single autosomal locus (Wilson et al., 2001). Ewes that are carriers of the FecB gene have an increase in ovulation rate .9 to 1.8 ova and an increase in litter size of up to one lamb per ewe lambing (Willingham et al., 2000).

It has been shown that AMH has been found in 94% of females regardless of bodyweight or age (Lahoz et al., 2012). However, concentrations were highly variable between animals, ranging from 0 (n=5, females that did not test positive for AMH) to 590 pg/ml. AMH concentration in blood plasma was not correlated with age or live weight (Lahoz et al., 2012). AMH plasma concentration was shown to not be correlated with the occurrence of the ovulation in response to equine chorionic gonadotropin (eCG), but not with the number of ovulations when only considering ovulating ewes (Lahoz et al., 2012). FecB positive ewes express and secrete lower levels of AMH from the granulosa cells, resulting in

in low AMH blood concentrations, despite the high numbers of AMH secreting follicles in the ovary (Estienne et al., 2015).

Ewes with the FecB ovulate more ova per ovulation, but also produce a smaller corpus luteum (CL) per ovum ovulated (Gonzalez et al., 2004). Older sheep that are FecB positive maintained a higher ovulation rate than sheep that are FecB negative [4.2 ± 0.6 corpora lutea (CL), respectively; $P>0.05$] and in younger sheep both ovulation and CL (4.7 ± 0.3 vs.6.9 ±0.7 mm and 12.8 ± 0.5 vs. 16.7 ± 0.8mm, respectively) were smaller than non-carrier ewes ($P<0.05$; Gonzalez et al., 2004). Follicle stimulating hormone (FSH), LH, P4, and PGF2 hormone patterns do not differ between Booroola and non-Booroola genotypes. Follicle Stimulating Hormone concentration and pattern during periovulatory period are similar between genotypes, although the average level is 2 to 3 times higher than that reported in young ewes (Gonzalez et al., 2004). With these studies it can be concluded that AMH could be a possible predictor to fertility characteristics. Anti-Mullerian hormone has been shown to have a positive correlation with ovulation, follicular reserve, and AFC's. Anti-Mullerian hormone is shown to inhibit the primordial follicles from reaching ovulation and preventing follicle reserve exhaustion. The FecB gene is known for increasing prolificacy and a ewe that has one copy of the FecB gene will have one lamb more than a ewe without the gene. Anti-Mullerian hormone concentration levels in FecB ewes tend to be lower than that of ewes without the gene.

MATERIALS AND METHODS

Two flocks of Rambouillet ewes at the Texas A&M University Barnhart Experiment Ranch in Irion County in West Texas were used. The ewes in these experiments were the offspring of rams that were heterozygous for the FecB gene and thus on average are genetically similar except half of the ewes have one copy of this gene (B/+) and the other half have no copies of the gene (+/+). The B/+ ewes should have approximately 1 additional lamb compared to ewes with no copies of this gene. This results in a unique population of ewes for testing the hypothesis of this experiment.

Group 1 consists of 199 maiden ewes that were born in 2012. Group 2 consist of 116 ewe lambs that were born in 2013. These ewes were kept on rangelands and were supplemented as needed. Anti-Mullerian Hormone profiles were examined for possible relationships to fertility characteristics such as puberty, pregnancy, and fecundity, within each group. Each ewe had blood samples, weights, and pregnancy data collected throughout the study. An ALOKA SSD-500V ultrasound with a 3.5 MHz transducer was used for trans-abdominal pregnancy determination examination. Pregnancy data was collected at d 60 of gestation. Blood samples were taken for AMH and PSPB. Pregnancy specific protein B was utilized to confirm pregnancy status. The Equine and Ovine AMH Immunoassay from Ansh Labs in Webster, TX was utilized for AMH concentrations of each sample. All animal procedures were performed in accordance with AUP 2013 18A as approved by the Agriculture Animal Care and Use Committee.

Group 1

The flock of 199 yearling ewes had blood samples collected and were weighed on September 30th, 2013 and the ewes were exposed to the rams on October 9th 2013 as the start

of the breeding season. Blood samples were taken with venous blood collection tubes using vacutainer blood tubes and 20 guage x 1-inch needles and blood collected via jugular vein. The blood samples were taken for the purpose of testing for AMH. Blood samples were put on ice until the sample could be centrifuged. Each sample, within 24 h of collection, was centrifuged for 45mins to separated red blood cells from the blood serum. The serum was then drawn from the blood collection tube and deposited into a serum tube. The serum was then frozen and shipped to Texas Veterinary Diagnostic Lab in College Station, Texas to be analyzed for AMH and PSPB. The Equine and Ovine AMH ELISA immunoassay was utilized for the detection of AMH concentrations within each sample of serum. Every collection was handled the same way. On November 20th 2013 rams were removed from the flock and the breeding season ended. On December 20th 2013, blood samples were collected for AMH and Pregnancy Specific Protein B (PSPB). Pregnancy data was obtained via trans-abdominal ultrasound and also to obtain number of fetuses for each ewe at mid-gestation.

Group 2

The flock of 108 ewe lambs had the first blood samples taken and were weighed, at 6 months of age, on September 25th, 2013. Samples from this collection date were obtained for AMH concentrations. The beginning of the breeding season was started when ewes were exposed to the rams on October 6th 2014. The next blood sample for this group was December 18th, 2014. Blood samples, weights, and ultrasound data was recorded. The blood samples were taken for AMH as well as PSPB. Ultrasound was used to find pregnant, non-pregnant, and multiple fetuses data at mid-gestation.

Equine and Ovine AMH ELISA Immunoassay

The Equine and Ovine AMH ELISA is a quantitative three-step sandwich type immunoassay. The first step is to serially dilute Calibrators and unknown samples added to AMH antibody coated micro titer wells and incubated (AnshLabs, 2014). After the first incubation and washing, the wells are incubated with biotinylated AMH solution. After second incubation and washing the wells are incubated with streptavidin horseradish peroxide conjugate (SHRP) solution (AnshLabs, 2014). After the third incubation and washing, the wells are incubated with substrate solution followed by an acidic stopping solution. In principle, the antibody-biotin conjugate binds to the streptavidin-enzyme conjugate (AnshLabs, 2014). The antibody-antigen-biotin conjugate-SHRP complex bound to the well is detected by enzyme substrate reaction. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm as primary test filter and 630 nm as a reference filter (AnshLabs, 2014). The absorbance measured is directly proportional to the concentration of AMH in the samples and calibrators (AnshLabs, 2014).

Statistics

The concentration of AMH was analyzed using SAS PROC GLM. The model included an effect for FecB genotype and body weight as covariates. A model with fertility, as assessed by ultrasound, as the dependent variable was used to evaluate the significance of using AMH as a predictor for fertility. Fertility was coded as one for pregnant and zero for nonpregnant. The model for fertility included FecB genotype, and body weight and AMH as covariates.

Ewes were categorized as to how many lambs were visible via ultrasound as zero, one, or greater than one. This measure of litter size was a dependent variable in a model that included FecB genotype and body weight and AMH as covariates.

RESULTS

Group 1

Average BW for the September 25th 2013 sample was 66.04 ± 5.8 kg. The mean BW for the December 20th 2013 sample was 70.34 ± 5.75 kg. Average AMH concentration for September 25th, 2013 sample was .034 ± 0.044 ng/ml (Table 1). Average AMH concentration for December 20th, 2013 sample was .204 ± 0.12 ng/ml (Table 1). Factors that were tested in PROC GLM that could have an effect on AMH concentration were BW and genotype. Body weight had no significant effect on AMH concentration (P= 0.15). Genotype +/+ ewes had significantly higher AMH concentration prior to ram exposure (P=0.002) and also at mid-gestation (P=0.02) than ewes with the genotype B/+ (Table 2).

Factors that could affect fertility were AMH and genotype. The fertility of both genotypes was similar (P=0.17). Anti-Mullerian hormone concentration before ram exposure and at mid-gestation did not show a significant effect on fertility (P=0.24; P=0.5). Fetal count did not have a significant effect on AMH concentrations when considering only pregnant ewes (P= 0.73).

Table 1: AMH Serum Concentrations and BW in Rambouillet Ewes Compared at Different Ages

	N	Age, mo.	BW, kg	AMH, ng/ml
Group 1	190	18	66.04 ± 5.8	.034 ± 0.04
	190	21	70.34 ± 5.75	.204 ± 0.12
Group 2	108	6	39.9 ± 4.9	0.042 ± 0.04
	103	21	56.7 ± 5.9	0.064 ± 0.06

*Group 1 ewes born 2012. Group 2 ewes born 2013.

Table 2: Genotype and AMH concentration compared at ram turn in and at mid-gestation.

	+/+ AMH concentration ng/ml ± SE	B/+ AMH concentration ng/ml ± SE	P Value
Sept. 30, 2013	.05 ± 0.004	.02 ± 0.004	P=0.002
Dec. 20, 2013	.23 ± 0.013	.18 ± 0.014	P=0.02

+/+ = Noncarriers of FecB
B/+ = One copy of FecB

Table 3: Genotype and AMH concentrations related to fertility in Group 1 and Group 2

	Group 1	Group 2
B/+	.91 ± 0.03	.81 ± .05
+/+	.95 ± 0.03	.79 ± .06
AMH ng/ml	.204± 0.117	0.06 ±.06
P Value	P= 0.33	P=0.77

Group 2

Average BW for the September 25th 2013 sample was 39.9 ± 4.9 kg. The average BW for the December 20th 2014 sample was 56.7 ± 5.9 kg. Average AMH concentration for September 25th, 2013 sample was 0.042 ± 0.04 ng/ml. Average AMH concentration for December 20th, 2014 sample was 0.064 ± 0.06 ng/ml (Table 1). Factors that were tested in the GLM procedure that could have an affect on AMH concentration were BW and genotype. Body weight showed to have no significant effect on AMH concentration (P=0.19). Ewes' genotyped +/+ had a significant higher AMH concentration as ewe lambs at 6 months old (P=0.002) and also at mid-gestation at 21 months of age (P=0.009) than ewes with the genotype B/+ (Table 4).

Fertility was measured using trans-abdominal ultrasound and confirmed using PSPB. Factors tested in the GLM procedure that could affect fertility were AMH concentration and genotype. Genotype did not have a significant effect on fertility using ultrasound or PSPB data (P=0.77; P=0.94). Anti-Mullerian concentration at mid gestation did not have a significant effect on fertility (P=0.11). Fetal counts were significantly related to AMH concentration (P= 0.05). Ewes carrying singles had an average AMH concentration of 0.05 ± 0.008 and ewes carrying multiples had an AMH concentration of 0.08 ± 0.01.

Table 4: AMH concentration by genotype at 6 months of age and at mid-gestation.

	+/+ AMH ng/ml ± SE	B/+ AMH ng/ml ± SE	P Value
Sept 2013	0.05 ± .005	.03 ± .006	P=0.002
Dec. 2014	0.08 ± .007	0.05 ± .008	P=0.009

Table 5: Fetal counts when compared to AMH concentration at mid-gestation

	Singles	Multiples	P Value
AMH ng/ml ± SE	0.05 ± 0.008	0.08 ± 0.01	P=0.04

DISCUSSION

Based on limited research of the comparison of AMH concentration and pregnancy rates, there are few reports comparable to the present study. However, according to Ribeiro et al. (2014) AMH concentration in plasma has been used as a marker for ovarian reserve and also has been associated with fertility traits in dairy cows. In the present study it is shown that the FecB gene has a significant relationship with AMH concentration. It was shown in Estienne et al., (2015) that AMH concentrations were reduced in B/B ewes when compared to +/+ ewes ($P < .001$). In the present study it was shown that B/+ ewes have a significantly lower AMH concentration when compared to +/+ ewes. This relationship could possibly be a predictor for prolificacy in a later study if lambing data would be analyzed. According to Lahoz et al., 2012 AMH concentration was not significantly correlated with BW. Similarly was show in both groups in the present study that BW did not have an effect on AMH concentrations.

It was shown in the present study that AMH concentration was not related to pregnancy rate when comparing all animals within a group. In Lahoz et al. (2012) prepubertal AMH concentration was higher in ewes that became pregnant at the first mating than that of those that did not conceive ($P<0.05$). In this study we found that 11% of ewes confirmed pregnant by PSPB were confirmed open via ultrasound. This could be an indicator that ewes that were bred during the first cycle might have a higher AMH concentration than that of ewes that conceive during the second estrus cycle. It was shown that in Group 2 that fetal count was related to AMH concentration, but did not show a significant relationship in Group 1. This could possibly be a relationship with age as well as AMH concentration. This would need to be repeated in a new study to confirm data. According to Lahoz et al. (2012)

age or live weight are not related to AMH concentration or fertility. Body weight did not have an effect on AMH concentration in this study but body condition score was not taken and that could have been an effect on conception rate.

In group 2, ultrasound results were 81% confirmed pregnant and PSPB results confirmed 92% pregnant. For future studies, to find earliness of conception, a PSPB sample could be taken at the end of each estrus cycle to confirm pregnancy. Ultrasound data needs to be recorded at mid-gestation for ewes that became pregnant during the first cycle and for the ewes that became pregnant during the second cycle.

IMPLICATIONS

The present study has not shown that AMH concentrations have a significant effect on short term reproduction. However, other studies involving different species have shown to have positive relationships with antral follicle counts, ovarian reserve, and in-vitro fertilization. The present study can be used as a model for new studies. If future studies would include a long term study involving the type of sheep in the present study, then the possibility of finding a relationship between AMH concentrations would be greater.

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